

CHROMOSOME ABNORMALITIES IN AN INDIVIDUAL OF CHORTHIPPUS LONGICORNIS (ACRIDIDAE)

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CHROMOSOME abnormalities in the Acrididae so far described have had to do mainly with the so-called fusion of rod-shaped chromosomes to form V-shaped ones, with an accompanying reduction of chromosome number. The 17-chromosome forms of the Truxalinae; Chorthippus, Chloaltis, Chrysochraon, and the 19-chromosome form Neopodismopsis (*Rothfels*, unpublished) have, no doubt, been derived from twenty-three-chromosome forms in this way. They illustrate the so-called "Robertson Law." In all probability, these V-chromosomes originated not by simple fusions but by very unequal reciprocal translocations leading to the elimination of short segments from both rod chromosomes concerned and including only a single centromere from one of the two fusing chromosomes. The minute chromosome formed as the second product of the reciprocal translocation must then have been eliminated. The multiple chromosomes of Hesperotettix and Mermiria described by McCLUNG (1927) also appear to have been derived in this way (see MULLER 1940, p. 223).

Another type of chromosome modification which appears to be common in western species of Circotettix and Trimerotropis (CAROTHERS 1931, KING 1923, HELWIG 1933) is a shift in the centromere from near the end to a submedian position. This gives rise to metacentric chromosomes without any reduction in number or increase in size. In these genera, as in Hesperotettix and Mermiria, the modification has not become fixed and varies from individual to individual.

The change in position of the centromere in Circotettix and Trimerotropis has been ascribed to (a) a pericentric inversion, or (b) a lateral shift in the centromere (HELWIG, 1933, WHITE 1940). As pointed out by WHITE, the former alternative appears to be the more probable but, as far as I am aware, no direct observational evidence has been advanced in support of either.

Evidence of paracentric inversions in the Orthoptera in the form of bridges, is not abundant in the literature, but the phenomenon is a common one and such inversions must be practically universal in grasshopper species. Heteromorphic bivalents due to unequal lengths have also been found in a number of forms (see WHITE 1945, p. 108) and must, I believe, be almost equally widespread.

When we look for evidence in the Acrididae for another common source of abnormality, viz. reciprocal translocations, we find it confined to reports by CAROTHERS (1931), WHITE (1940) and HELWIG (1942). In these cases the evidence was confined to first meiotic metaphase and anaphase configurations. On the basis of its repeated occurrence in more than one year in the same

locality, WHITE considered the cases reported by him as systemic in nature, but the grounds for this view appear to be insufficient. The percentage of multivalents (quadrivalents) found by him in any one individual was low, and investigations in this laboratory indicate that recurrent non-systemic translocations in grasshoppers are by no means so rare as has been assumed.

MATERIAL AND METHODS

The material here reported upon is from a male of *Chorthippus longicornis* collected, along with many others, in the neighborhood of Port Carling, Muskoka, Canada, in August 1945. All material collected at that time was being used in preliminary tests on the effects of dilute aqueous solutions of colchicine on mitosis and meiosis, the solutions being injected into the abdomens of the grasshoppers, and the testes fixed and stained at intervals up to five days after the treatment. It is not proposed to report on the effects of the colchicine treatment at this time, except in so far as it affected the material from this one individual. As in all the plant material so far reported upon, the treatment led to complete inhibition of spindle formation, so that if the nuclei were not already in anaphase no chromosome separation took place. The result was a huge accumulation of spermatogonial and first meiotic metaphases. Meiotic prophases, more especially early and late diplophases, also showed up very clearly. Mitotic and meiotic anaphases were almost absent and owing to the marked shortening and thickening of the chromosomes were of no use for study. The shortening and thickening of the chromosomes at metaphase and the failure of the V-shaped chromosomes to bend at the centromere is a further characteristic result of treatment with colchicine, as can be seen in Plate 2, figure 2 and Plate 4, figures 11 and 12.

The testis was dissected out into Ringer's solution, the fatty tissue surrounding it removed and the whole testis fixed in modified Levitsky's chromformol (equal parts of one percent chromic acid and ten percent formalin). It was then stained *in toto* by the Feulgen method, individual tubules were

PLATE I

Explanation of Figures

FIGURE 1.—Pachyphase configuration showing pairing relationships of the four chromosomes involved in the multivalent, its individual elements being labeled.

FIGURE 2.—Early diplophase configuration showing, below, the result of a crossover in the region of the pericentric inversion and, above, association of the chromosome $1^{3,7}$ with a 7^1 chromosome.

FIGURE 3.—Early diplophase of same stage as figure 2. The homologous ends of the two arms below have formed a subterminal chiasma which, on terminalization, will form a ring such as shown at the bottom of multivalents reproduced in figures 4, 6 and 7.

FIGURE 4.—Late diplophase. Complete complement showing a univalent above and to right.

FIGURE 5.—Late diplophase. A complement showing univalent above and to right. One of the small bivalents appears to have been attached to or hidden by the X chromosome below to left.

FIGURES 6, 7, 8.—Late diplophase—different forms of the multivalent. In figures 6 and 8 chromosome 7^1 is included; in figure 7 it is not.

Magnification in all cases approximately 1600 diameters.



PLATE I

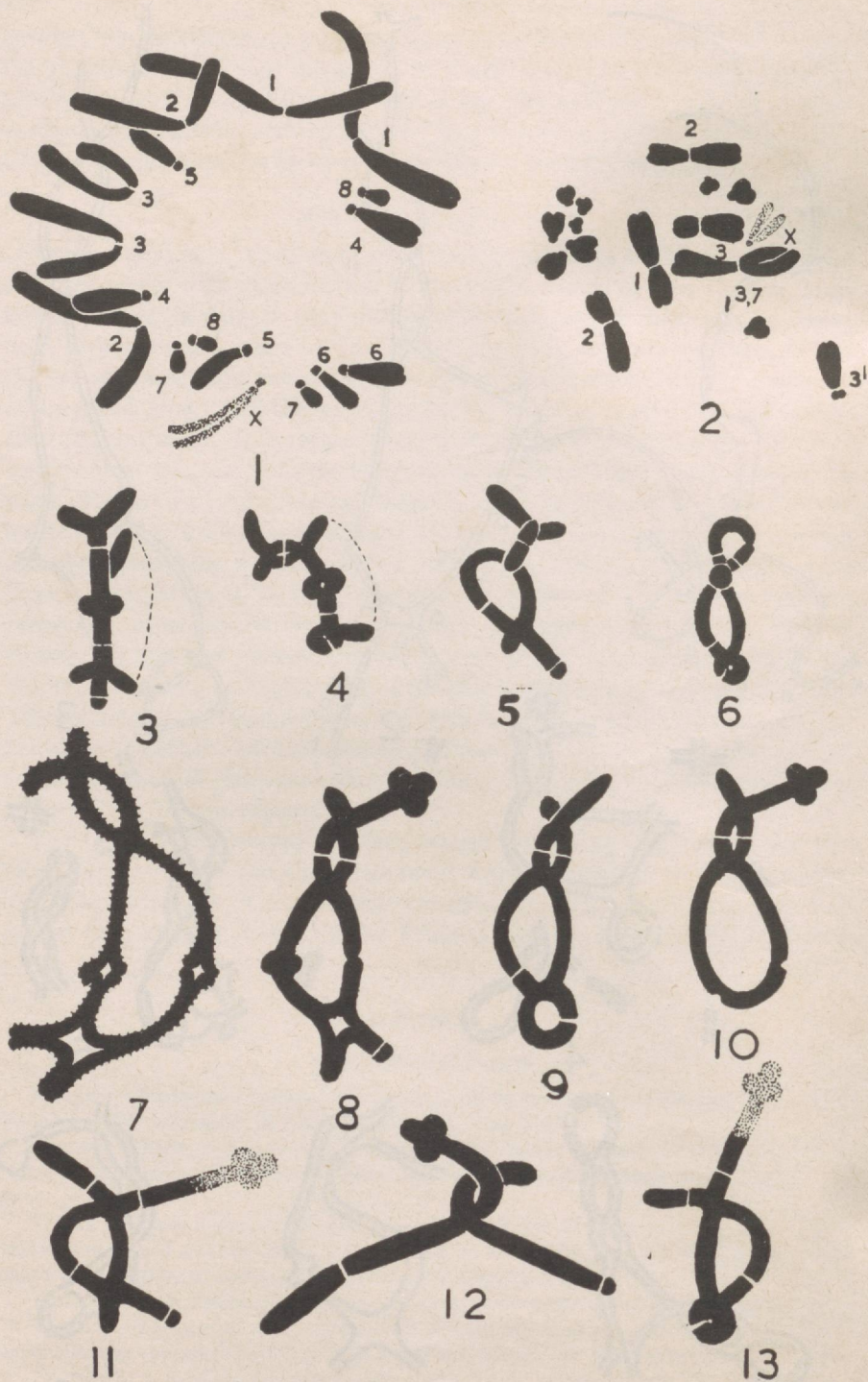


PLATE 2

squashed on slides in 50 percent acetic acid, and the slides were transferred through dioxane to dioxane balsam.

OBSERVATIONS

Before proceeding to a consideration of the abnormalities observed, it is necessary to refer briefly to the normal chromosomes, although their morphology has been described by previous authors. An examination of Plate 2, figure 1 and Plate 4, figure 10, reproducing respectively an early and a late spermatogonial metaphase, shows the presence of three metacentric chromosome pairs which can be readily distinguished from one another and which are numbered 1-3 in descending order of length. Following these in descending order are five pairs of acrocentric chromosome pairs (4-8 inclusive) plus, of course, a single X chromosome.

A study of spermatogonial metaphases in the individual under examination revealed a marked change in two of the metacentric chromosomes, one belonging to pair 1 and the other to pair 3. The longer of these, labeled $1^{3,7}$ in Plate 2, figure 2, and Plate 4, figures 11 and 12, is longer than the normal chromosome 1 and has its centromere approximating more closely to the median position. This means that, although both arms have been lengthened, the shorter one has been elongated more than the longer one.

The altered chromosome 3, labeled in the same figures as 3^1 , is somewhat shorter than its partner and has its centromere in an acrocentric instead of the

PLATE 2

Explanation of Figures

FIGURE 1.—A normal early spermatogonial metaphase showing the different types of chromosomes.

FIGURE 2.—Spermatogonial metaphase in the abnormal individual with only the long pairs labeled. Irregular arrangement, shortening of chromosomes and failure to form V-shape is due to colchicine treatment.

FIGURES 3 and 4.—First meiotic metaphase multivalents showing failure of chiasma formation between chromosome 3^1 and chromosome 1.

FIGURE 5.—Multivalent at first metaphase showing result of chiasma formation at one side of the inversion loop. Chromosome 7^1 not involved here.

FIGURE 6.—Multivalent at first metaphase showing terminal rings due to formation of sub-terminal chiasmata between (a) 7^1 and 7 and (b) the two homologous ends due to chiasma in inversion.

FIGURE 7.—Late diplophase multivalent showing incomplete chromatid below and unterminalized chiasmata at sides.

FIGURE 8.—First metaphase multivalent showing equal open arms below and chiasma between $1^{3,7}$ and 7^1 above.

FIGURE 9.—First metaphase multivalent with ring below and no association of 7^1 above.

FIGURE 10.—First metaphase multivalent showing result of failure of chiasma formation in the inversion but retention of terminal chiasma distal to inversion.

FIGURES 11 and 13.—First metaphase multivalents showing negative heteropycnosis in the region of the $1^{3,7}$ and 7^1 association.

FIGURE 12.—First metaphase multivalent in which chiasmata in the chromosome 3 part of the configuration are entirely lacking.

Magnification in all cases approximately 1600 diameters.

usual metacentric position. At first sight one might be tempted to ascribe this apparent change of position of the centromere to a deletion in the shorter arm. However, the long arm of this chromosome is distinctly longer than the long arm of its normal partner, and pairing relations and diplophase configurations, to be described, show clearly that the centromere shift is due to a pericentric inversion asymmetric with reference to the centromere.

The pairing relationships of these translocated chromosomes $1^{3,7}$ and 3^1 with their normal partners are shown clearly in Plate 1, figure 1 and Plate 3, figure 1, both representing the same structure. In the lower right corner of each figure is shown the typical inversion loop with the approximate position of the two centromeres of the No. 3 chromosomes, marked at *c* in Plate 1, figure 1. This position has been obtained by using the relative lengths of the two arms of 3 and 3^1 respectively at premeiotic metaphase. Gene loci are indicated in the normal third chromosome by the letters A-I, and corresponding gene loci of translocated chromosome 3^1 are marked in the region where the two synapse. The univalents are already split at this stage but they are shown single in Plate 1, figure 1, to simplify the drawing.

Plate 1, figure 2, and Plate 3, figure 3 both show the same early diplophase configuration where there has been a crossing over in the region of the inversion between gene loci G and H. As a result there has been formed, as one of the products, an incomplete chromatid in which the gene locus I has been duplicated, an inevitable result of a cross-over in this type of inversion. A comparison of Plate 1, figure 2 with figure 1 of the same plate will show that the position given to the gene loci in this region is approximately correct, although the loci seem too far apart relative to their distance apart in the region A-G. This must be due to a greater stretching of the chromonemata in the region G-I owing to the rather heavy pressure applied in squashing. Farther up can be seen two further chiasmata, one between chromosome $1^{3,7}$ and chromosome 3, the other between chromosome 1 and chromosome 3^1 . The latter is already almost terminalized.

The presence of similar ends in the deficient chromatid leads to the possibility of chiasma formation in the region of the I locus as is shown in Plate 1, figure 3 and Plate 3, figure 4. Terminalization of this chiasma gives the terminal ring shown in Plate 3, figures 5 and 7 and in many of the later diplophases (Plate 2, figures 4, 6 and 9, and Plate 3, figures 8 and 9, Plate 4, figure 4). Metaphase configurations showing the same ring are reproduced in Plate 1, figures 4, 6, 8 Plate 2, figure 13, and Plate 4, figure 7. The open arm structure which results from a failure of chiasma formation in this region is shown for early prophase in Plate 1, figure 2 and Plate 3, figures 2 and 3, for later prophase in Plate 1, figure 7, Plate 2, figures 5, 8 and 11 and Plate 4, figures 5, 6 and 8. The relative lengths of the two arms may vary greatly depending upon just where, in the inversion, the chiasma has been formed. A chiasma far from the middle of the inversion loop is responsible for the extreme asymmetry shown in Plate 2, figure 5 and Plate 3, figure 3. It is also evident in such configurations as are shown in Plate 1, figures 4 and 8, and Plate 2, figure 6, where the position of the centromere shows the asymmetry.

Where chiasma formation fails in the inversion but is present in the region distal to it, one obtains, on terminalization, a large ring at the bottom of the number 3 chromosome end of the configuration. Illustrations of this are given in Plate 2, figure 10 and Plate 4, figure 9. Where chiasmata fail entirely in this part of the complex, a figure such as that shown in Plate 2, figure 12 results. These two last types are comparatively rare, the former having been found in about ten percent, the latter in about one percent of the configurations examined. Either one of these configurations would produce viable gametes in 50 percent of the cases as far as the genes in chromosomes 1 and 3 are concerned.

As will be noted on an examination of first metaphase figures in the plates, the position of the centromere is strikingly evident in the case of all of the four long chromosomes involved, as a more or less complete unstained transverse band on the chromosomes. This enables one to identify the different members of the configuration with great exactitude and to establish the fact that the relationship to each other in the multivalent remains constant. It also enables one to fix approximately the position of terminalized chiasmata where, as at first metaphase, they are usually not visible.

When we come to chiasma formation between the number 3 and the number 1 chromosomes, at least one is always present, thus ensuring the formation of a quadrivalent. Usually chiasmata are formed on both sides, that is between chromosome $1^{3,7}$ and chromosome 3 on the one side and chromosome 1 and chromosome 3^1 on the other. These lateral chiasmata are shown in most of the first meiotic prophase figures as for example Plate 3, figures 2-8. In some cases the chiasma on one side becomes terminalized at an early stage of diplophase as in figure 2, in others both persist to a comparatively late stage as in figure 6. At times terminalization is incomplete at first metaphase as in Plate 2, figure 8 to the left and Plate 3, figure 8 to the left.

Where the chiasma fails on one side we obtain configurations like those illustrated in Plate 2, figures 3 and 4, the arms in which chiasma formation has failed here being joined by dotted lines. The exact position of terminalized chiasmata can commonly not be identified at metaphase but occasionally it can be made out as in Plate 2, figure 8. Here the chiasma between chromosome $1^{3,7}$ and chromosome 3 has remained unterminalized.

Turning to the part of the configuration involving a translocation between a chromosome 1 and one of the short acrocentric chromosomes, measurements indicate with a high degree of probability that this latter is a number 7 chromosome. It is also apparent that the number 1 chromosome which is involved in this translocation has also taken part in the 1-3 translocation. The drawing, from which Plate 1 figure 1 has been reduced, showed a difference in length, taken from a point in close synapsis, of 2.5 cm between this chromosome and the other number 1 chromosome. This is about the same length as the normal chromosome number 7 at the same magnification and approximately the same stage of pachyphase. The accompanying diagram gives the approximate relative lengths of the translocated pieces and of the resultant translocated chromosomes.

It has not been possible to identify any chromosome corresponding to the

small 7^1 chromosome represented in the diagram, which must have been one of the products of an unequal reciprocal translocation. As will be seen from an examination of Plate 4, figure 10, in a spermatogonial metaphase of a normal individual, the 17 chromosomes characteristic of the species can be made out

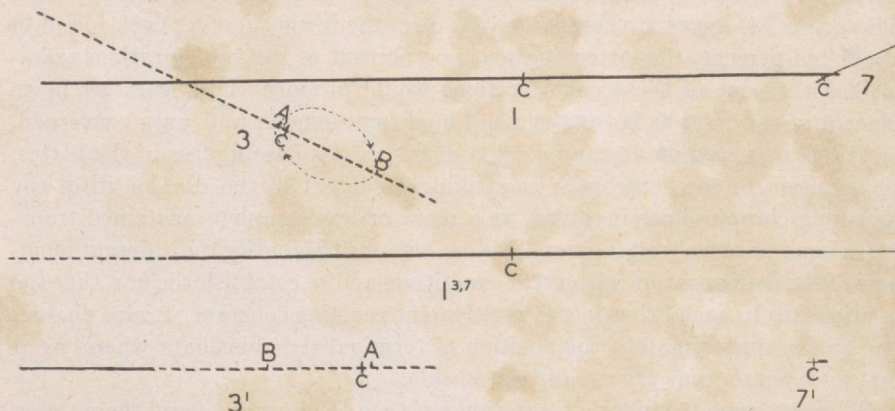


Diagram showing the main translocations between chromosomes 1, 3, and 7 with products. The centromere is marked by C, the inverted section in chromosome 3 by AB.

without any difficulty. On the other hand, figures 11 and 12 on the same plate, which show similar metaphases from the abnormal individual, show only 16 chromosomes or 15 plus X. One of the short chromosomes must be present in the haploid number. Also if we examine complete first meiotic division nuclei, we find that first meiotic prophases and metaphases also appear to contain a univalent (upper right in Plate 1, figures 4 and 5) in those cases where there is no evidence of synapsis of the short chromosome with the end of chromosome $1^{3,7}$. In case of synapsis there is one less unit than in cases where no synapsis occurs. The total number of units is seven instead of the eight that are other-

PLATE 3

Explanation of Figures

FIGURE 1.—Compare with Plate 1, figure 1.

FIGURE 2.—Early prophase showing a complete complement. One of the short bivalents looks as if attached to an arm of the multivalent at extreme top but really lies beneath it.

FIGURE 3.—Compare with Plate 1, figure 2.

FIGURE 4.—Compare with Plate 1, figure 3.

FIGURES 5 and 7. Early prophase multivalents showing complete rings below owing to terminalization of subterminal chiasma.

FIGURE 6.—Late diplophase showing complete complement. The univalent 7^1 is above to left. Compare with Plate 2, figure 7.

FIGURE 8.—Late diplophase showing complete complement. The univalent 7^1 is above to right. Compare with Plate 1, figure 4.

FIGURE 9.—Late diplophase showing complete complement. Univalent 7^1 attached to right of multivalent, thus reducing separate units from 8 to 7. Compare figure 8.

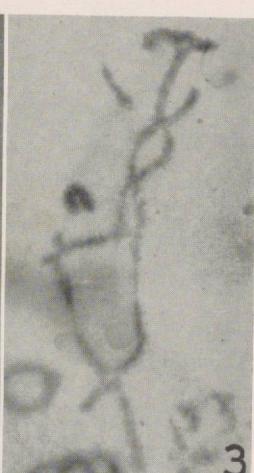
Magnification in all cases approximately 950 diameters.



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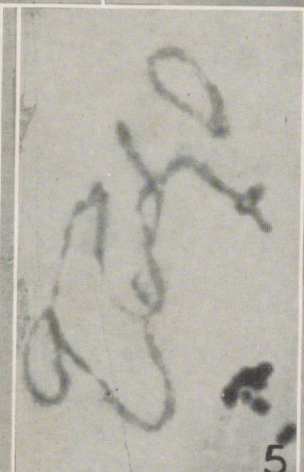
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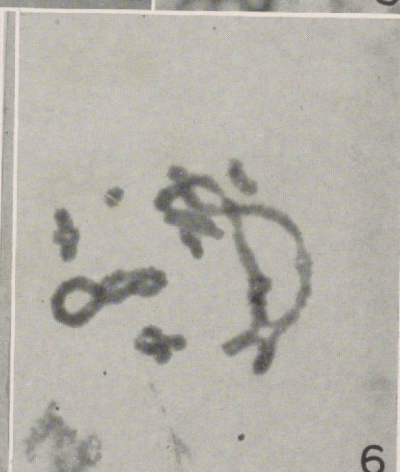
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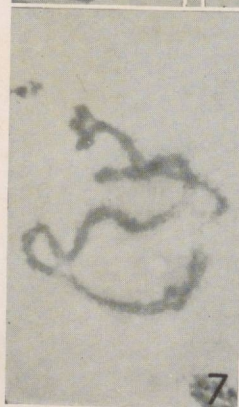
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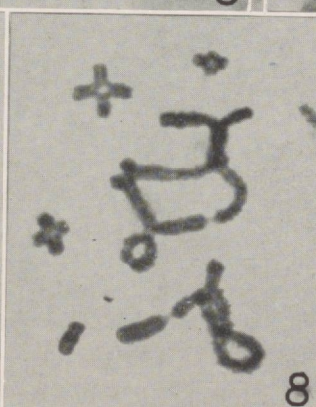
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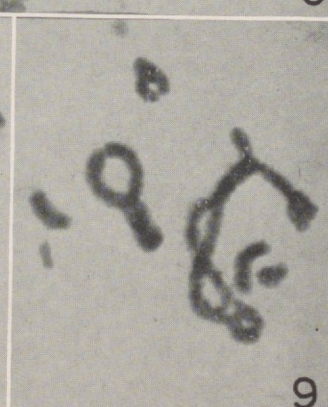
6



7



8



9

PLATE 3

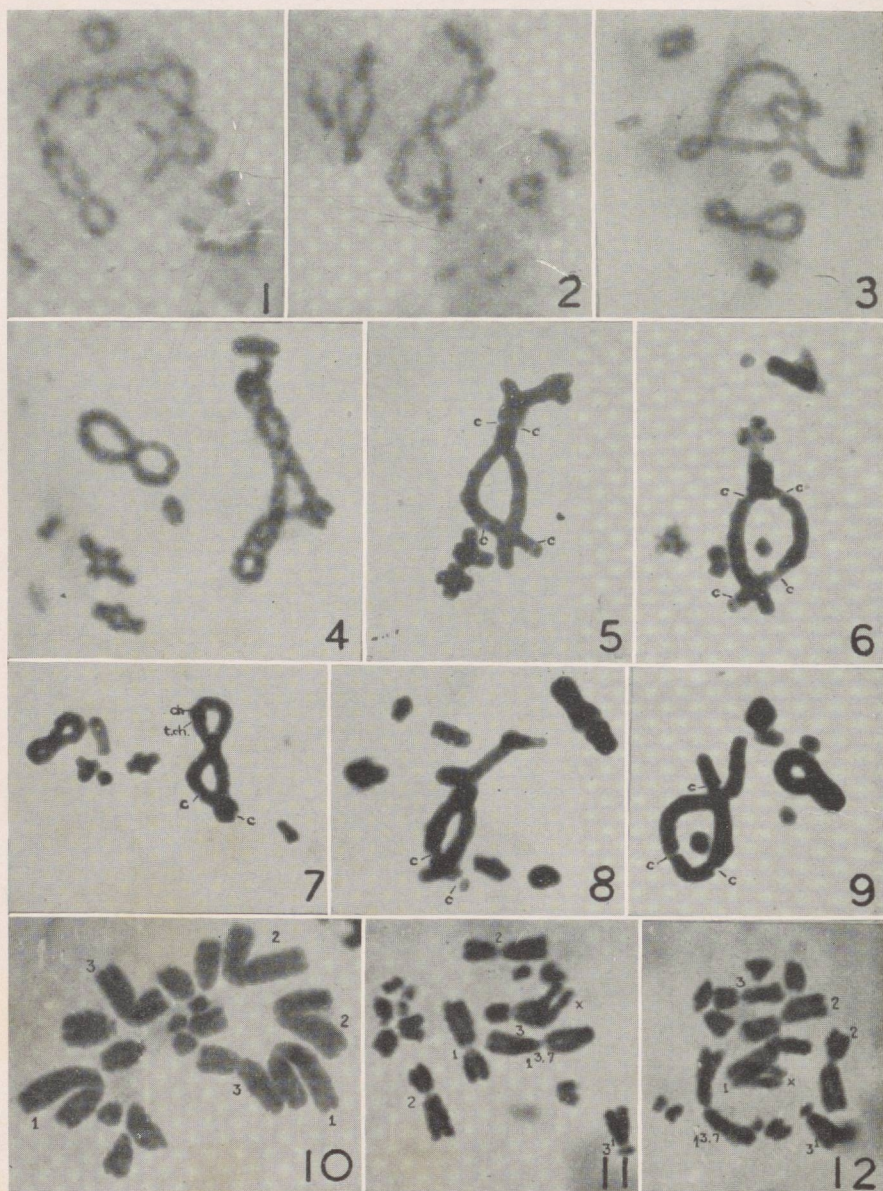


PLATE 4

wise present or the nine that are present at first meiotic metaphase in normal forms.

At first it would appear that the separate short chromosome involved at the top of configurations illustrated in Plate 1, figure 2 and Plate 3, figures 2, 3, 5 and 7 must be a normal chromosome 7. However, when we examine Plate 2, figure 6 and Plate 4, figure 7 we find a terminalized chiasma forming a ring. As pairing and chiasma formation take place only between homologous parts there must be at one end of the small chromosome 7, a small terminal portion of chromosome 1, or at the end of chromosome 1 a small section from the short arm of chromosome 7. It is true, if we postulate the presence of the small translocated chromosome represented in the diagram, its interpolation in the sequence would give us a sexavalent, but, as already stated, there is no evidence of the retention of this small chromosome. Plate 4, figure 7 especially shows the terminal chiasma between the small chromosome 7 and chromosome 1 (t. ch), and close above it the unterminalized chiasma (ch) between the chromosome 7 and chromosome 1^{3,7}. We are, therefore, forced to conclude that this configuration is a quinquevalent not a sexavalent.

It seems to me probable that a chromosome 7 carries this last translocation 1. The portion of the chromosome lost by such a translocation would have to be in the short arm of chromosome 7 which, as it does not extend much beyond the centromere, is likely to be relatively inert genically. The loss of an end section of a chromosome 1 would be likely to lead to greater genic disturbance and a less viable chromosome. Further, it seems necessary to postulate that this translocation took place in the opposite parent of the heterozygote from the one providing the original translocations between chromosomes 1, 3 and 7. The chromosome 7¹ found in the nuclei of this individual therefore consists of a long arm and centromere derived from chromosome 7 and a short segment from the end of chromosome 1 which replaces an end segment of the short arm of 7. We are, however, not at the end of

PLATE 4

Explanation of Figures

FIGURES 1-3.—Mid-diplophase nuclei in which the whole complement can be made out with some degree of certainty.

FIGURE 4.—Late diplophase—compare with Plate 1, figure 6.

FIGURE 5.—First metaphase multivalent showing open arms below and attachment of 7¹ above.

FIGURE 6.—First metaphase multivalent showing negative heteropynosis above.

FIGURE 7.—Complete complement at first metaphase. Multivalent shows ring above and beneath. Compare with Plate 2, figure 6.

FIGURE 8.—Complete complement at first metaphase.

FIGURE 9.—First metaphase multivalent showing failure of chiasma formation within the inversion. Compare Plate 2, figure 10.

FIGURE 10.—Normal late spermatogonial metaphase with metacentric chromosomes numbered (photo K. ROTHFELS). Compare with Plate 2, figure 1.

FIGURES 11 and 12.—Spermatogonial metaphases with normal and translocated long chromosomes numbered. Compare with Plate 2, figure 2.

Magnification in all cases approximately 950 diameters.

the phenomena connected with the 1-7 association. As is evident from Plate 2, figures 11 and 13 and Plate 4, figure 6, the 7-portion of $1^{3,7}$ and the 7^1 may show negative heteropycnosis. This is not universally the case as can be seen from Plate 2, figures 5, 10 and 12. It was not confined to any particular testicular tubule, so it is hard to see how any difference in environmental conditions or any difference of fixation could account for this phenomenon. For the present I shall have to leave it as an uninterpreted observation. This heteropycnosis is, at least partially, due to a less compact coiling of the chromonemata as is evidenced by the greater length of the end involved in these figures as compared to the length in comparable figures such as Plate 2, figure 8.

DISCUSSION

The various configurations which have been described and illustrated must, in almost every case, yield a high proportion of non-viable gametes. Reciprocal translocation between chromosomes 1 and 3 would, in itself, lead to 50 percent non-viability unless some mechanism is present favoring proper disjunction. Lack of first meiotic anaphase figures prevents any decision on this point. Failure of pairing of chromosome 7^1 with chromosome $1^{3,7}$, which was apparent in about 50 percent of the 318 nuclei examined for this purpose, would almost certainly lead to failure of proper distribution of the univalent. Finally, crossing over in the inversion region of chromosome 3 would lead to duplications and deficiencies in two of the four chromatids concerned. This was found to take place in 89 percent of the configurations examined for the purpose. The only types that are free from this disability are those in which ends near the inversion are free, as in Plate 2, figure 12 representing one percent of the total or those in which a chiasma is formed only distal to the inversion as in Plate 2, figure 10, and Plate 4, figure 9. These represented about ten percent of the total.

Notwithstanding these facts, a large number of the spermatozoa present in the preparations appeared to be quite normal. There were a greater number of giant spermatozoa than are usually found, but they still formed a small percentage of the total. I need hardly state that the individual itself showed no morphological peculiarity such as would strike one in an ordinary examination. As stated, I was studying not morphological differences but the effect of colchicine and could hardly believe my eyes when the chromosome abnormalities here described met them.

All the abnormalities described above, with the possible exception of the persisting 7^1 translocation, must have arisen in the parental generation of this complex heterozygote. The various chromosomal changes need not have arisen during one mitotic cycle. They may have been scattered over a series of successive cell divisions. They must, however, almost certainly have arisen during one generation for all the changes with the exception of the final 7^1 translocation are such as to cause very heavy reduction in fertility. The heterozygote cannot have arisen as a result of a chance union of two distinct cytological races differing in gene arrangement as may happen for example in *Drosophila azteca* (DOBZHANSKY and SOCOLOV 1939). It must be emphasized that this was a single individual collected in an area from which large numbers have been examined.

All the other individuals collected and studied cytologically, whether they had been treated with colchicine or not, showed no more striking hybrid character than the presence of paracentric inversions as evidenced by first and second anaphase bridges. This type of abnormality is likely to be found in almost any grasshopper population if one deals with any considerable number of individuals.

The pericentric inversion here described is, as far as I am aware, the first one that has been properly documented by a study of different meiotic stages. The presence of pericentric inversions has, for the most part, been inferred from a shift in the position of the centromere observable at mitotic metaphase as in *Ichthyophis* (SESHACHAR 1939) or at first meiotic metaphase and anaphase as in *Circotettix* and *Trimerotropis* (CAROTHERS 1921, KING 1923, HELWIG 1929). Nothing has been reported on the mode of transmission of the heteromorphic chromosomes in the first case but, in the case of *Circotettix* and *Trimerotropis*, the presence of these inversions apparently does not affect fertility. MULLER (1940) has suggested that this is due to a localization of chiasmata in chromosome sections outside the region of inversion and more particularly in subterminal regions. The bases for this conclusion are the facts that the products of disjunction at first meiotic anaphase are normal and that no reduction of viability occurs. It should be emphasized, however, that, in each of the cases cited, the shift of the centromere has been from an acrocentric to a metacentric position and, as the short arm of the acrocentric chromosomes in these forms is relatively very short, there will be a very short terminal region left in which a chiasma outside the inversion could be formed. This will greatly reduce the chances of chiasma formation in this region. At metaphase, in these forms, the bivalents are usually maintained by a terminal chiasma at the end of the long arm farthest removed from the centromere. However, an examination of Carother's figures (1921, text figure A2 and 4) shows that a chiasma may be formed in the short arm of the chromosomes presumed to contain the inversion, while HELWIG (1929, p. 5) states that this happens quite frequently. In his later paper (1942) HELWIG expresses doubt that a change in position is due to a pericentric inversion on the ground that irradiation, which would be expected to produce an inversion effect never does so. As apparently no chiasmata are formed in the short arm when these chromosomes have their normal acrocentric structure, the observations suggest a lateral shift of the centromere rather than an inversion. If we postulate the shift of a small piece of the chromosome immediately surrounding the centromere, this would not seriously affect chiasma formation, but would affect the direction in which some chiasmata would move in terminalization. Such a shift would involve three breaks in the chromosome instead of the two required for an inversion but it has been shown in this paper that three breaks are not an excessive number to expect in grasshopper material. CAROTHERS, HELWIG and KING unfortunately do not give any figures illustrating stages before first meiotic metaphase but the metaphase figures show clearly that chiasmata are by no means localized in the normal chromosomes of species of *Circotettix* and *Trimerotropis* in general. It seems clear that the whole

question of centromere shift in grasshoppers, and in other forms where it has been reported, requires reexamination, using a more suitable cytological technique than has heretofore been employed.

SUMMARY

1. An individual of *Chorthippus longicornis* has been found to be heterozygous for no less than four chromosome abnormalities.

2. One of the longest chromosomes (chromosome 1) shows a reciprocal translocation with one of the third longest chromosomes (chromosome 3) at one end and a translocation involving most of one of the second shortest chromosomes (chromosome 7) at the other end. The resulting chromosome $1^{3,7}$ is considerably longer than its partner with its centromere occupying a more nearly median position.

3. The translocated chromosome 3 (3^1) contains, in addition, a pericentric inversion leading to a shift of the centromere from a submedian (metacentric) to a subterminal (acrocentric) position. Previous centromere shifts in grasshoppers (*Trimerotropis*, *Circotettix*) and amphibia which have been accounted for by the presence of a pericentric inversion have been from a subterminal to a submedian position. Moreover, the presence of a pericentric inversion has been postulated solely on the basis of a change in position of the centromere and the possibility of a lateral shift without any inversion is not excluded. In the present case early pachyphase and diplotyphase configurations show conclusively that the pericentric inversion is involved. Whereas, in the grasshoppers *Trimerotropis* and *Circotettix*, if the postulated pericentric inversions exist, crossing over apparently does not take place within them, in the *Chorthippus* individual, crossing over in the inversion has taken place in approximately 90 percent of the configurations examined.

4. The reciprocal translocation which involves chromosome 1 and chromosome 7 has led to the transfer of most of chromosome 7 to the end of chromosome 1 and must have resulted in the formation of a minute chromosome involving the centromeric region of 7 and a very small piece from the end of 1. This minute chromosome appears to have disappeared, probably in one of the two parents from which this individual was derived. There is no evidence of it either in the premeiotic metaphase, in the multivalent or as a univalent during the first meiotic division. However, there is present a translocated 7^1 chromosome which must have arisen in the opposite parent of the heterozygote, by an exchange of a minute terminal part of the short arm of a chromosome 7 with a short terminal section of chromosome 1. This allows for pairing on the one side with the 7 end of chromosome $1^{3,7}$ and on the other side with the end of untranslocated chromosome 1 to form the distal ring found in about 25 percent of the configurations studied. Where chromosome 7^1 does not pair on either side, it appears as a univalent. Where it pairs solely with chromosome $1^{3,7}$ it forms a cross at the end of this chromosome. This has been found in about 28 percent of the cases examined. Not infrequently a negative heteropycnosis is found involving the translocated end of chromosome $1^{3,7}$ and chromosome 7^1 forming the cross. No explanation for this can, at present, be suggested, but it

is at least partly, due to a less contracted coiling of the chromonemata in this region.

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CHROMOSOME ABNORMALITIES IN AN INDIVIDUAL OF CHORTHIPPUS LONGICORNIS (ACRIDIDAE)

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CHROMOSOME abnormalities in the Acrididae so far described have had to do mainly with the so-called fusion of rod-shaped chromosomes to form V-shaped ones, with an accompanying reduction of chromosome number. The 17-chromosome forms of the Truxalinae; Chorthippus, Chloea, and Chrysochaon, and the 19-chromosome form Neopodisma (Rothfels, unpublished) have, no doubt, been derived from twenty-three-chromosome forms in this way. They illustrate the so-called "Robertson Law." In all probability, these V-chromosomes originated not by simple fusions but by very unequal reciprocal translocations leading to the elimination of short segments from both rod chromosomes concerned and including only a single centromere from one of the two fusing chromosomes. The minute chromosome formed as the second product of the reciprocal translocation must then have been eliminated. The multiple chromosomes of Hesperotettix and Mermiria described by McCLUNG (1927) also appear to have been derived in this way (see MULLER 1940, p. 223).

Another type of chromosome modification which appears to be common in western species of Circotettix and Trimerotropis (CAROTHERS 1931, KING 1923, HELWIG 1933) is a shift in the centromere from near the end to a submedian position. This gives rise to metacentric chromosomes without any reduction in number or increase in size. In these genera, as in Hesperotettix and Mermiria, the modification has not become fixed and varies from individual to individual.

The change in position of the centromere in Circotettix and Trimerotropis has been ascribed to (a) a pericentric inversion, or (b) a lateral shift in the centromere (HELWIG, 1933, WHITE 1940). As pointed out by WHITE, the former alternative appears to be the more probable but, as far as I am aware, no direct observational evidence has been advanced in support of either.

Evidence of paracentric inversions in the Orthoptera in the form of bridges, is not abundant in the literature, but the phenomenon is a common one and such inversions must be practically universal in grasshopper species. Heteromorphic bivalents due to unequal lengths have also been found in a number of forms (see WHITE 1945, p. 108) and must, I believe, be almost equally widespread.

When we look for evidence in the Acrididae for another common source of abnormality, viz. reciprocal translocations, we find it confined to reports by CAROTHERS (1931), WHITE (1940) and HELWIG (1942). In these cases the evidence was confined to first meiotic metaphase and anaphase configurations. On the basis of its repeated occurrence in more than one year in the same

locality, WHITE considered the cases reported by him as systemic in nature, but the grounds for this view appear to be insufficient. The percentage of multivalents (quadrivalents) found by him in any one individual was low, and investigations in this laboratory indicate that recurrent non-systemic translocations in grasshoppers are by no means so rare as has been assumed.

MATERIAL AND METHODS

The material here reported upon is from a male of *Chorthippus longicornis* collected, along with many others, in the neighborhood of Port Carling, Muskoka, Canada, in August 1945. All material collected at that time was being used in preliminary tests on the effects of dilute aqueous solutions of colchicine on mitosis and meiosis, the solutions being injected into the abdomens of the grasshoppers, and the testes fixed and stained at intervals up to five days after the treatment. It is not proposed to report on the effects of the colchicine treatment at this time, except in so far as it affected the material from this one individual. As in all the plant material so far reported upon, the treatment led to complete inhibition of spindle formation, so that if the nuclei were not already in anaphase no chromosome separation took place. The result was a huge accumulation of spermatogonial and first meiotic metaphases. Meiotic prophase, more especially early and late diplophases, also showed up very clearly. Mitotic and meiotic anaphases were almost absent and owing to the marked shortening and thickening of the chromosomes were of no use for study. The shortening and thickening of the chromosomes at metaphase and the failure of the V-shaped chromosomes to bend at the centromere is a further characteristic result of treatment with colchicine, as can be seen in Plate 2, figure 2 and Plate 4, figures 11 and 12.

The testis was dissected out into Ringer's solution, the fatty tissue surrounding it removed and the whole testis fixed in modified Levitsky's chrom-formol (equal parts of one percent chromic acid and ten percent formalin). It was then stained *in toto* by the Feulgen method, individual tubules were

PLATE I

Explanation of Figures

FIGURE 1.—Pachyphase configuration showing pairing relationships of the four chromosomes involved in the multivalent, its individual elements being labeled.

FIGURE 2.—Early diplophase configuration showing, below, the result of a crossover in the region of the pericentric inversion and, above, association of the chromosome $1^{2,7}$ with a 7^1 chromosome.

FIGURE 3.—Early diplophase of same stage as figure 2. The homologous ends of the two arms below have formed a subterminal chiasma which, on terminalization, will form a ring such as shown at the bottom of multivalents reproduced in figures 4, 6 and 7.

FIGURE 4.—Late diplophase. Complete complement showing a univalent above and to right.

FIGURE 5.—Late diplophase. A complement showing univalent above and to right. One of the small bivalents appears to have been attached to or hidden by the X chromosome below to left.

FIGURES 6, 7, 8.—Late diplophase—different forms of the multivalent. In figures 6 and 8 chromosome 7^1 is included; in figure 7 it is not.

Magnification in all cases approximately 1600 diameters.



PLATE I

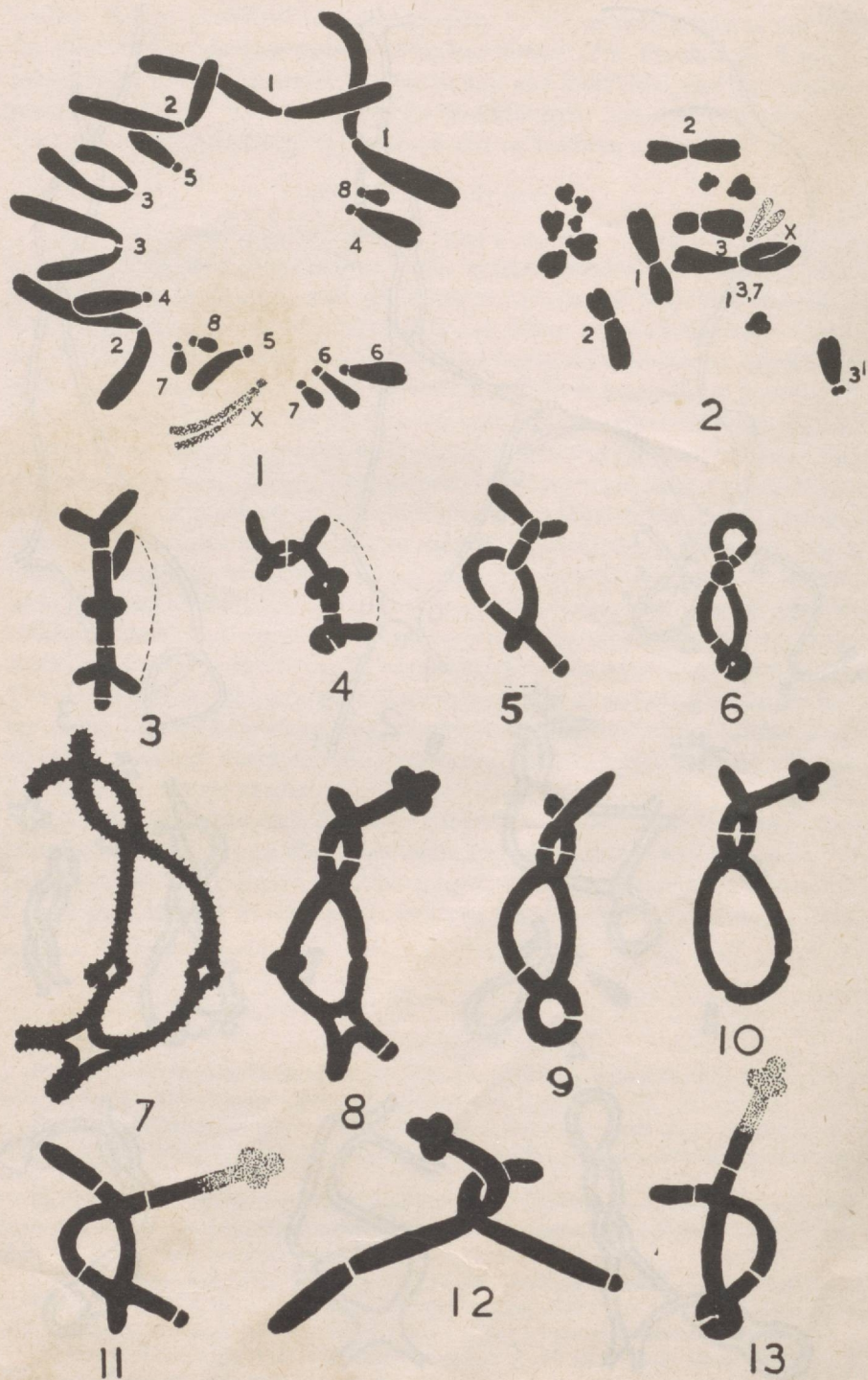


PLATE 2

squashed on slides in 50 percent acetic acid, and the slides were transferred through dioxane to dioxane balsam.

OBSERVATIONS

Before proceeding to a consideration of the abnormalities observed, it is necessary to refer briefly to the normal chromosomes, although their morphology has been described by previous authors. An examination of Plate 2, figure 1 and Plate 4, figure 10, reproducing respectively an early and a late spermatogonial metaphase, shows the presence of three metacentric chromosome pairs which can be readily distinguished from one another and which are numbered 1-3 in descending order of length. Following these in descending order are five pairs of acrocentric chromosome pairs (4-8 inclusive) plus, of course, a single X chromosome.

A study of spermatogonial metaphases in the individual under examination revealed a marked change in two of the metacentric chromosomes, one belonging to pair 1 and the other to pair 3. The longer of these, labeled $1^{3,7}$ in Plate 2, figure 2, and Plate 4, figures 11 and 12, is longer than the normal chromosome 1 and has its centromere approximating more closely to the median position. This means that, although both arms have been lengthened, the shorter one has been elongated more than the longer one.

The altered chromosome 3, labeled in the same figures as 3^1 , is somewhat shorter than its partner and has its centromere in an acrocentric instead of the

PLATE 2

Explanation of Figures

FIGURE 1.—A normal early spermatogonial metaphase showing the different types of chromosomes.

FIGURE 2.—Spermatogonial metaphase in the abnormal individual with only the long pairs labeled. Irregular arrangement, shortening of chromosomes and failure to form V-shape is due to colchicine treatment.

FIGURES 3 and 4.—First meiotic metaphase multivalents showing failure of chiasma formation between chromosome 3^1 and chromosome 1.

FIGURE 5.—Multivalent at first metaphase showing result of chiasma formation at one side of the inversion loop. Chromosome 7^1 not involved here.

FIGURE 6.—Multivalent at first metaphase showing terminal rings due to formation of sub-terminal chiasmata between (a) 7^1 and 7 and (b) the two homologous ends due to chiasma in inversion.

FIGURE 7.—Late diplophase multivalent showing incomplete chromatid below and unterminalized chiasmata at sides.

FIGURE 8.—First metaphase multivalent showing equal open arms below and chiasma between $1^{3,7}$ and 7^1 above.

FIGURE 9.—First metaphase multivalent with ring below and no association of 7^1 above.

FIGURE 10.—First metaphase multivalent showing result of failure of chiasma formation in the inversion but retention of terminal chiasma distal to inversion.

FIGURES 11 and 13.—First metaphase multivalents showing negative heteropycnosis in the region of the $1^{3,7}$ and 7^1 association.

FIGURE 12.—First metaphase multivalent in which chiasmata in the chromosome 3 part of the configuration are entirely lacking.

Magnification in all cases approximately 1600 diameters.

usual metacentric position. At first sight one might be tempted to ascribe this apparent change of position of the centromere to a deletion in the shorter arm. However, the long arm of this chromosome is distinctly longer than the long arm of its normal partner, and pairing relations and diplophase configurations, to be described, show clearly that the centromere shift is due to a pericentric inversion asymmetric with reference to the centromere.

The pairing relationships of these translocated chromosomes $1^{3.7}$ and 3^1 with their normal partners are shown clearly in Plate 1, figure 1 and Plate 3, figure 1, both representing the same structure. In the lower right corner of each figure is shown the typical inversion loop with the approximate position of the two centromeres of the No. 3 chromosomes, marked at *c* in Plate 1, figure 1. This position has been obtained by using the relative lengths of the two arms of 3 and 3^1 respectively at premeiotic metaphase. Gene loci are indicated in the normal third chromosome by the letters A-I, and corresponding gene loci of translocated chromosome 3^1 are marked in the region where the two synapse. The univalents are already split at this stage but they are shown single in Plate 1, figure 1, to simplify the drawing.

Plate 1, figure 2, and Plate 3, figure 3 both show the same early diplophase configuration where there has been a crossing over in the region of the inversion between gene loci G and H. As a result there has been formed, as one of the products, an incomplete chromatid in which the gene locus I has been duplicated, an inevitable result of a cross-over in this type of inversion. A comparison of Plate 1, figure 2 with figure 1 of the same plate will show that the position given to the gene loci in this region is approximately correct, although the loci seem too far apart relative to their distance apart in the region A-G. This must be due to a greater stretching of the chromonemata in the region G-I owing to the rather heavy pressure applied in squashing. Farther up can be seen two further chiasmata, one between chromosome $1^{3.7}$ and chromosome 3, the other between chromosome 1 and chromosome 3^1 . The latter is already almost terminalized.

The presence of similar ends in the deficient chromatid leads to the possibility of chiasma formation in the region of the I locus as is shown in Plate 1, figure 3 and Plate 3, figure 4. Terminalization of this chiasma gives the terminal ring shown in Plate 3, figures 5 and 7 and in many of the later diplophases (Plate 2, figures 4, 6 and 9, and Plate 3, figures 8 and 9, Plate 4, figure 4). Metaphase configurations showing the same ring are reproduced in Plate 1, figures 4, 6, 8 Plate 2, figure 13, and Plate 4, figure 7. The open arm structure which results from a failure of chiasma formation in this region is shown for early prophase in Plate 1, figure 2 and Plate 3, figures 2 and 3, for later prophase in Plate 1, figure 7, Plate 2, figures 5, 8 and 11 and Plate 4, figures 5, 6 and 8. The relative lengths of the two arms may vary greatly depending upon just where, in the inversion, the chiasma has been formed. A chiasma far from the middle of the inversion loop is responsible for the extreme asymmetry shown in Plate 2, figure 5 and Plate 3, figure 3. It is also evident in such configurations as are shown in Plate 1, figures 4 and 8, and Plate 2, figure 6, where the position of the centromere shows the asymmetry.

Where chiasma formation fails in the inversion but is present in the region distal to it, one obtains, on terminalization, a large ring at the bottom of the number 3 chromosome end of the configuration. Illustrations of this are given in Plate 2, figure 10 and Plate 4, figure 9. Where chiasmata fail entirely in this part of the complex, a figure such as that shown in Plate 2, figure 12 results. These two last types are comparatively rare, the former having been found in about ten percent, the latter in about one percent of the configurations examined. Either one of these configurations would produce viable gametes in 50 percent of the cases as far as the genes in chromosomes 1 and 3 are concerned.

As will be noted on an examination of first metaphase figures in the plates, the position of the centromere is strikingly evident in the case of all of the four long chromosomes involved, as a more or less complete unstained transverse band on the chromosomes. This enables one to identify the different members of the configuration with great exactitude and to establish the fact that the relationship to each other in the multivalent remains constant. It also enables one to fix approximately the position of terminalized chiasmata where, as at first metaphase, they are usually not visible.

When we come to chiasma formation between the number 3 and the number 1 chromosomes, at least one is always present, thus ensuring the formation of a quadrivalent. Usually chiasmata are formed on both sides, that is between chromosome 1^{3.7} and chromosome 3 on the one side and chromosome 1 and chromosome 3¹ on the other. These lateral chiasmata are shown in most of the first meiotic prophase figures as for example Plate 3, figures 2-8. In some cases the chiasma on one side becomes terminalized at an early stage of diplotene as in figure 2, in others both persist to a comparatively late stage as in figure 6. At times terminalization is incomplete at first metaphase as in Plate 2, figure 8 to the left and Plate 3, figure 8 to the left.

Where the chiasma fails on one side we obtain configurations like those illustrated in Plate 2, figures 3 and 4, the arms in which chiasma formation has failed here being joined by dotted lines. The exact position of terminalized chiasmata can commonly not be identified at metaphase but occasionally it can be made out as in Plate 2, figure 8. Here the chiasma between chromosome 1^{3.7} and chromosome 3 has remained unterminalized.

Turning to the part of the configuration involving a translocation between a chromosome 1 and one of the short acrocentric chromosomes, measurements indicate with a high degree of probability that this latter is a number 7 chromosome. It is also apparent that the number 1 chromosome which is involved in this translocation has also taken part in the 1-3 translocation. The drawing, from which Plate 1 figure 1 has been reduced, showed a difference in length, taken from a point in close synapsis, of 2.5 cm between this chromosome and the other number 1 chromosome. This is about the same length as the normal chromosome number 7 at the same magnification and approximately the same stage of pachyphase. The accompanying diagram gives the approximate relative lengths of the translocated pieces and of the resultant translocated chromosomes.

It has not been possible to identify any chromosome corresponding to the

small 7^1 chromosome represented in the diagram, which must have been one of the products of an unequal reciprocal translocation. As will be seen from an examination of Plate 4, figure 10, in a spermatogonial metaphase of a normal individual, the 17 chromosomes characteristic of the species can be made out

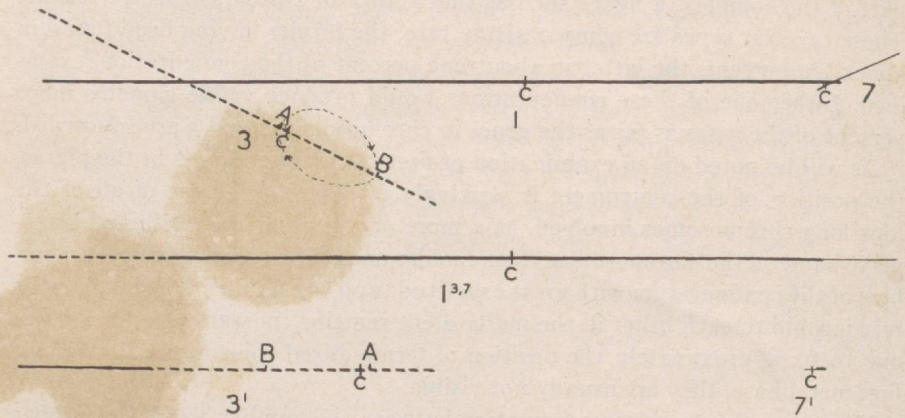


Diagram showing the main translocations between chromosomes 1, 3, and 7 with products. The centromere is marked by C, the inverted section in chromosome 3 by AB.

without any difficulty. On the other hand, figures 11 and 12 on the same plate, which show similar metaphases from the abnormal individual, show only 16 chromosomes or 15 plus X. One of the short chromosomes must be present in the haploid number. Also if we examine complete first meiotic division nuclei, we find that first meiotic prophase and metaphases also appear to contain a univalent (upper right in Plate 1, figures 4 and 5) in those cases where there is no evidence of synapsis of the short chromosome with the end of chromosome $1^{3,7}$. In case of synapsis there is one less unit than in cases where no synapsis occurs. The total number of units is seven instead of the eight that are other-

PLATE 3

Explanation of Figures

FIGURE 1.—Compare with Plate 1, figure 1.

FIGURE 2.—Early prophase showing a complete complement. One of the short bivalents looks as if attached to an arm of the multivalent at extreme top but really lies beneath it.

FIGURE 3.—Compare with Plate 1, figure 2.

FIGURE 4.—Compare with Plate 1, figure 3.

FIGURES 5 and 7. Early prophase multivalents showing complete rings below owing to terminalization of subterminal chiasma.

FIGURE 6.—Late diplophase showing complete complement. The univalent 7^1 is above to left. Compare with Plate 2, figure 7.

FIGURE 8.—Late diplophase showing complete complement. The univalent 7^1 is above to right. Compare with Plate 1, figure 4.

FIGURE 9.—Late diplophase showing complete complement. Univalent 7^1 attached to right of multivalent, thus reducing separate units from 8 to 7. Compare figure 8.

Magnification in all cases approximately 950 diameters.

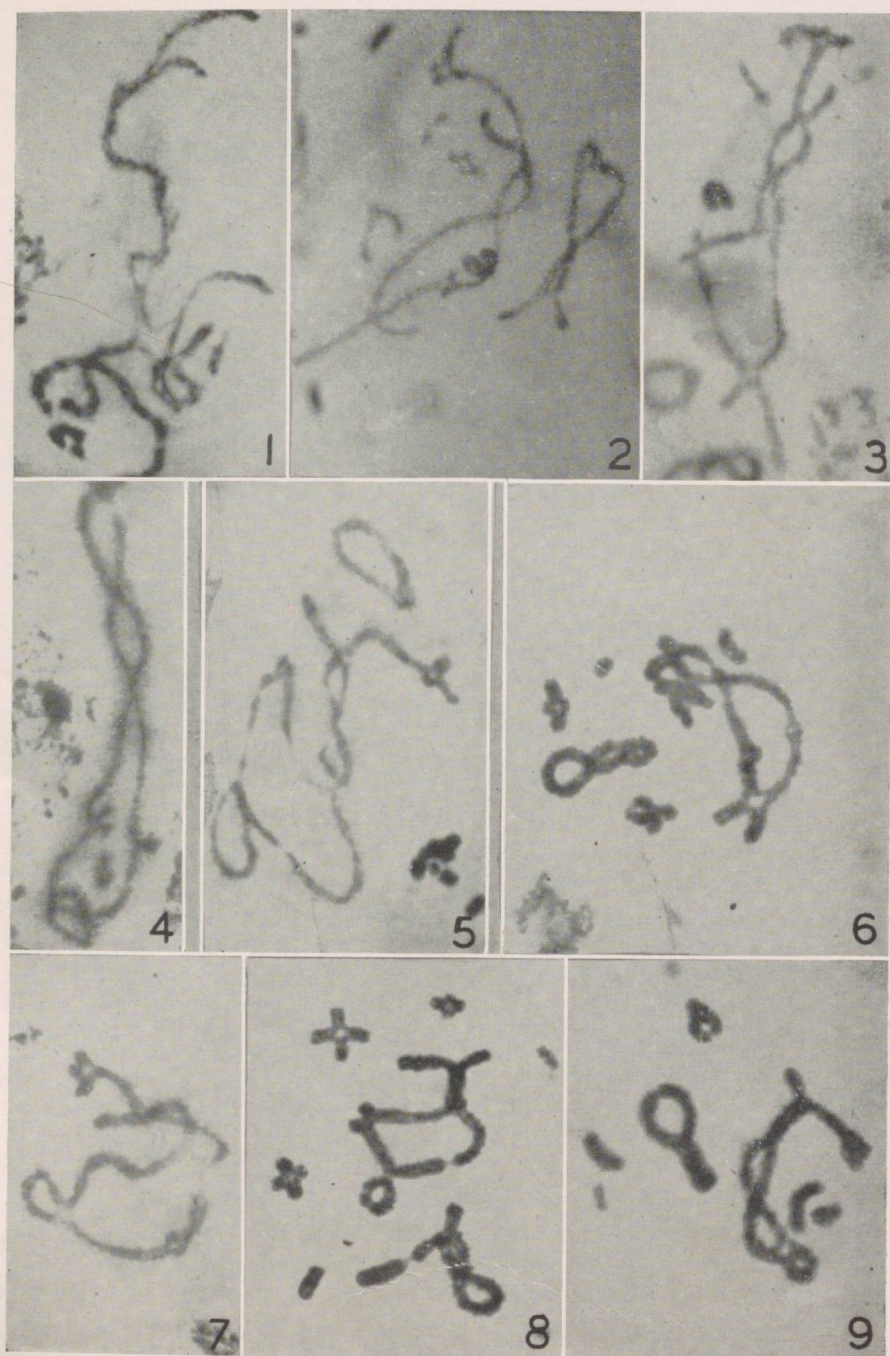


PLATE 3

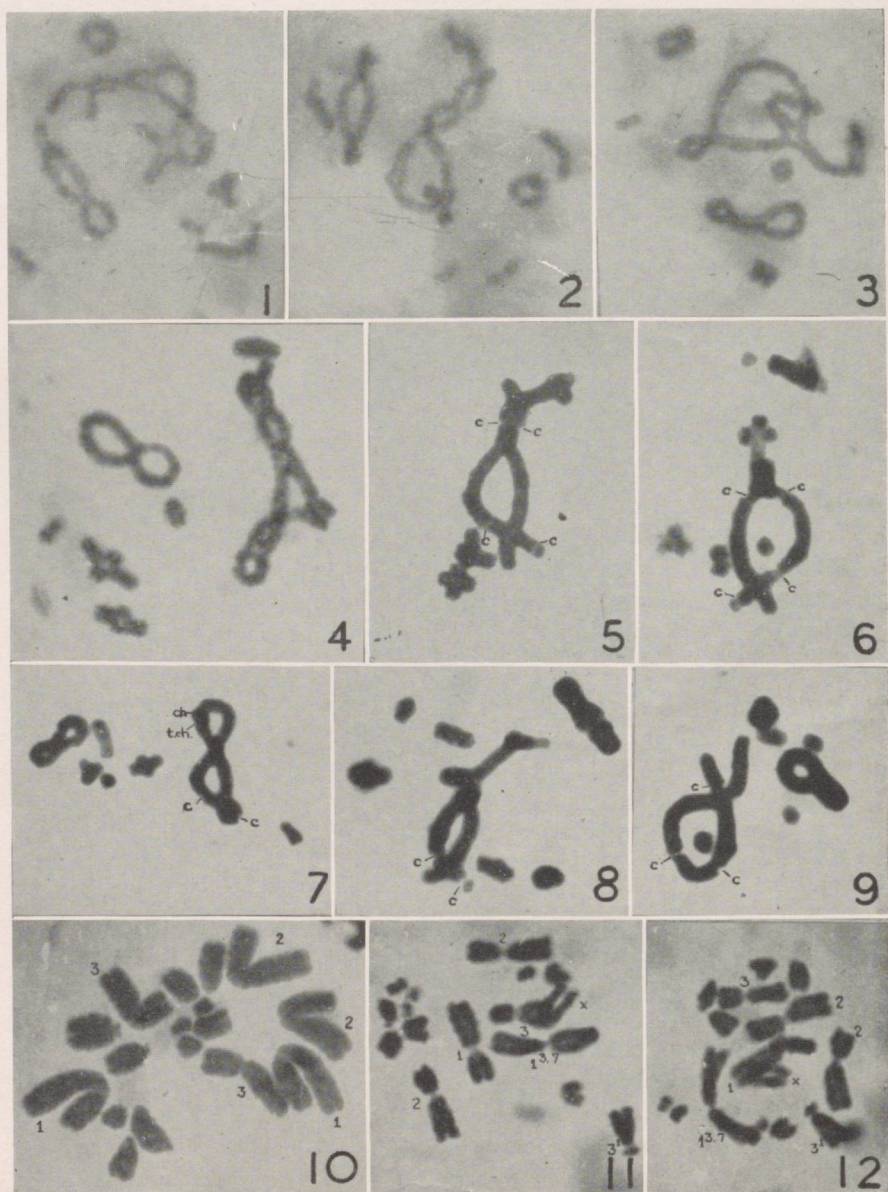


PLATE 4

wise present or the nine that are present at first meiotic metaphase in normal forms.

At first it would appear that the separate short chromosome involved at the top of configurations illustrated in Plate 1, figure 2 and Plate 3, figures 2, 3, 5 and 7 must be a normal chromosome 7. However, when we examine Plate 2, figure 6 and Plate 4, figure 7 we find a terminalized chiasma forming a ring. As pairing and chiasma formation take place only between homologous parts there must be at one end of the small chromosome 7, a small terminal portion of chromosome 1, or at the end of chromosome 1 a small section from the short arm of chromosome 7. It is true, if we postulate the presence of the small translocated chromosome represented in the diagram, its interpolation in the sequence would give us a sexavalent, but, as already stated, there is no evidence of the retention of this small chromosome. Plate 4, figure 7 especially shows the terminal chiasma between the small chromosome 7 and chromosome 1 (t. ch), and close above it the unterminalized chiasma (ch) between the chromosome 7 and chromosome 1^{3.7}. We are, therefore, forced to conclude that this configuration is a quinquevalent not a sexavalent.

It seems to me probable that a chromosome 7 carries this last translocation 1. The portion of the chromosome lost by such a translocation would have to be in the short arm of chromosome 7 which, as it does not extend much beyond the centromere, is likely to be relatively inert genically. The loss of an end section of a chromosome 1 would be likely to lead to greater genic disturbance and a less viable chromosome. Further, it seems necessary to postulate that this translocation took place in the opposite parent of the heterozygote from the one providing the original translocations between chromosomes 1, 3 and 7. The chromosome 7¹ found in the nuclei of this individual therefore consists of a long arm and centromere derived from chromosome 7 and a short segment from the end of chromosome 1 which replaces an end segment of the short arm of 7. We are, however, not at the end of

PLATE 4

Explanation of Figures

FIGURES 1-3.—Mid-diplophase nuclei in which the whole complement can be made out with some degree of certainty.

FIGURE 4.—Late diplophase—compare with Plate 1, figure 6.

FIGURE 5.—First metaphase multivalent showing open arms below and attachment of 7¹ above.

FIGURE 6.—First metaphase multivalent showing negative heteropycnosis above.

FIGURE 7.—Complete complement at first metaphase. Multivalent shows ring above and beneath. Compare with Plate 2, figure 6.

FIGURE 8.—Complete complement at first metaphase.

FIGURE 9.—First metaphase multivalent showing failure of chiasma formation within the inversion. Compare Plate 2, figure 10.

FIGURE 10.—Normal late spermatogonial metaphase with metacentric chromosomes numbered (photo K. ROTHFELS). Compare with Plate 2, figure 1.

FIGURES 11 and 12.—Spermatogonial metaphases with normal and translocated long chromosomes numbered. Compare with Plate 2, figure 2.

Magnification in all cases approximately 950 diameters.

the phenomena connected with the 1-7 association. As is evident from Plate 2, figures 11 and 13 and Plate 4, figure 6, the 7-portion of $1^{3.7}$ and the 7^1 may show negative heteropycnosis. This is not universally the case as can be seen from Plate 2, figures 5, 10 and 12. It was not confined to any particular testicular tubule, so it is hard to see how any difference in environmental conditions or any difference of fixation could account for this phenomenon. For the present I shall have to leave it as an uninterpreted observation. This heteropycnosis is, at least partially, due to a less compact coiling of the chromonemata as is evidenced by the greater length of the end involved in these figures as compared to the length in comparable figures such as Plate 2, figure 8.

DISCUSSION

The various configurations which have been described and illustrated must, in almost every case, yield a high proportion of non-viable gametes. Reciprocal translocation between chromosomes 1 and 3 would, in itself, lead to 50 percent non-viability unless some mechanism is present favoring proper disjunction. Lack of first meiotic anaphase figures prevents any decision on this point. Failure of pairing of chromosome 7^1 with chromosome $1^{3.7}$, which was apparent in about 50 percent of the 318 nuclei examined for this purpose, would almost certainly lead to failure of proper distribution of the univalent. Finally, crossing over in the inversion region of chromosome 3 would lead to duplications and deficiencies in two of the four chromatids concerned. This was found to take place in 89 percent of the configurations examined for the purpose. The only types that are free from this disability are those in which ends near the inversion are free, as in Plate 2, figure 12 representing one percent of the total or those in which a chiasma is formed only distal to the inversion as in Plate 2, figure 10, and Plate 4, figure 9. These represented about ten percent of the total.

Notwithstanding these facts, a large number of the spermatozoa present in the preparations appeared to be quite normal. There were a greater number of giant spermatozoa than are usually found, but they still formed a small percentage of the total. I need hardly state that the individual itself showed no morphological peculiarity such as would strike one in an ordinary examination. As stated, I was studying not morphological differences but the effect of colchicine and could hardly believe my eyes when the chromosome abnormalities here described met them.

All the abnormalities described above, with the possible exception of the persisting 7^1 translocation, must have arisen in the parental generation of this complex heterozygote. The various chromosomal changes need not have arisen during one mitotic cycle. They may have been scattered over a series of successive cell divisions. They must, however, almost certainly have arisen during one generation for all the changes with the exception of the final 7^1 translocation are such as to cause very heavy reduction in fertility. The heterozygote cannot have arisen as a result of a chance union of two distinct cytological races differing in gene arrangement as may happen for example in *Drosophila azteca* (DOBZHANSKY and SOCOLOV 1939). It must be emphasized that this was a single individual collected in an area from which large numbers have been examined.

All the other individuals collected and studied cytologically, whether they had been treated with colchicine or not, showed no more striking hybrid character than the presence of paracentric inversions as evidenced by first and second anaphase bridges. This type of abnormality is likely to be found in almost any grasshopper population if one deals with any considerable number of individuals.

The pericentric inversion here described is, as far as I am aware, the first one that has been properly documented by a study of different meiotic stages. The presence of pericentric inversions has, for the most part, been inferred from a shift in the position of the centromere observable at mitotic metaphase as in *Ichthyophis* (SESHACHAR 1939) or at first meiotic metaphase and anaphase as in *Circotettix* and *Trimerotropis* (CAROTHERS 1921, KING 1923, HELWIG 1929). Nothing has been reported on the mode of transmission of the heteromorphic chromosomes in the first case but, in the case of *Circotettix* and *Trimerotropis*, the presence of these inversions apparently does not affect fertility. MÜLLER (1940) has suggested that this is due to a localization of chiasmata in chromosome sections outside the region of inversion and more particularly in subterminal regions. The bases for this conclusion are the facts that the products of disjunction at first meiotic anaphase are normal and that no reduction of viability occurs. It should be emphasized, however, that, in each of the cases cited, the shift of the centromere has been from an acrocentric to a metacentric position and, as the short arm of the acrocentric chromosomes in these forms is relatively very short, there will be a very short terminal region left in which a chiasma outside the inversion could be formed. This will greatly reduce the chances of chiasma formation in this region. At metaphase, in these forms, the bivalents are usually maintained by a terminal chiasma at the end of the long arm farthest removed from the centromere. However, an examination of Carother's figures (1921, text figure A2 and 4) shows that a chiasma may be formed in the short arm of the chromosomes presumed to contain the inversion, while HELWIG (1929, p. 5) states that this happens quite frequently. In his later paper (1942) HELWIG expresses doubt that a change in position is due to a pericentric inversion on the ground that irradiation, which would be expected to produce an inversion effect never does so. As apparently no chiasmata are formed in the short arm when these chromosomes have their normal acrocentric structure, the observations suggest a lateral shift of the centromere rather than an inversion. If we postulate the shift of a small piece of the chromosome immediately surrounding the centromere, this would not seriously affect chiasma formation, but would affect the direction in which some chiasmata would move in terminalization. Such a shift would involve three breaks in the chromosome instead of the two required for an inversion but it has been shown in this paper that three breaks are not an excessive number to expect in grasshopper material. CAROTHERS, HELWIG and KING unfortunately do not give any figures illustrating stages before first meiotic metaphase but the metaphase figures show clearly that chiasmata are by no means localized in the normal chromosomes of species of *Circotettix* and *Trimerotropis* in general. It seems clear that the whole

question of centromere shift in grasshoppers, and in other forms where it has been reported, requires reexamination, using a more suitable cytological technique than has heretofore been employed.

SUMMARY

1. An individual of *Chorthippus longicornis* has been found to be heterozygous for no less than four chromosome abnormalities.

2. One of the longest chromosomes (chromosome 1) shows a reciprocal translocation with one of the third longest chromosomes (chromosome 3) at one end and a translocation involving most of one of the second shortest chromosomes (chromosome 7) at the other end. The resulting chromosome $1^{3,7}$ is considerably longer than its partner with its centromere occupying a more nearly median position.

3. The translocated chromosome 3 (3^1) contains, in addition, a pericentric inversion leading to a shift of the centromere from a submedian (metacentric) to a subterminal (acrocentric) position. Previous centromere shifts in grasshoppers (*Trimerotropis*, *Circotettix*) and amphibia which have been accounted for by the presence of a pericentric inversion have been from a subterminal to a submedian position. Moreover, the presence of a pericentric inversion has been postulated solely on the basis of a change in position of the centromere and the possibility of a lateral shift without any inversion is not excluded. In the present case early pachyphase and diplophase configurations show conclusively that the pericentric inversion is involved. Whereas, in the grasshoppers *Trimerotropis* and *Circotettix*, if the postulated pericentric inversions exist, crossing over apparently does not take place within them, in the *Chorthippus* individual, crossing over in the inversion has taken place in approximately 90 percent of the configurations examined.

4. The reciprocal translocation which involves chromosome 1 and chromosome 7 has led to the transfer of most of chromosome 7 to the end of chromosome 1 and must have resulted in the formation of a minute chromosome involving the centromeric region of 7 and a very small piece from the end of 1. This minute chromosome appears to have disappeared, probably in one of the two parents from which this individual was derived. There is no evidence of it either in the premeiotic metaphase, in the multivalent or as a univalent during the first meiotic division. However, there is present a translocated 7^1 chromosome which must have arisen in the opposite parent of the heterozygote, by an exchange of a minute terminal part of the short arm of a chromosome 7 with a short terminal section of chromosome 1. This allows for pairing on the one side with the 7 end of chromosome $1^{3,7}$ and on the other side with the end of untranslocated chromosome 1 to form the distal ring found in about 25 percent of the configurations studied. Where chromosome 7^1 does not pair on either side, it appears as a univalent. Where it pairs solely with chromosome $1^{3,7}$ it forms a cross at the end of this chromosome. This has been found in about 28 percent of the cases examined. Not infrequently a negative heteropycnosis is found involving the translocated end of chromosome $1^{3,7}$ and chromosome 7^1 forming the cross. No explanation for this can, at present, be suggested, but it

is at least partly, due to a less contracted coiling of the chromonemata in this region.

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